GP1644

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Practitioner's Docket No. MSU 4.1-406 **PATENT**

WP 7-5-00 #21

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Alberto L. Mendoza
Application No.: 09 /082,112 Group No.: 1644
Filed: 1998 May 20 Examiner: S. Turner
For: METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

Assistant Commissioner for Patents
Washington, D.C. 20231

TRANSMITTAL OF APPEAL BRIEF
(PATENT APPLICATION—37 C.F.R. § 1.192)

1. Transmitted herewith, in triplicate, is the APPEAL BRIEF in this application, with respect to the Notice of Appeal filed on April 27, 2000.

NOTE: "Appellant must, within two months from the date of the notice of appeal under § 1.191 or within the time allowed for reply to the action from which the appeal was taken, if such time is later, file a brief in triplicate. . . " 37 C.F.R. § 1.192(a) (emphasis added).

2. STATUS OF APPLICANT

This application is on behalf of

- ☐ other than a small entity.
- ☒ a small entity.

A statement:

- ☐ is attached.
- ☒ was already filed.

3. FEE FOR FILING APPEAL BRIEF

Pursuant to 37 C.F.R. § 1.17(c), the fee for filing the Appeal Brief is:

☒	small entity	$150.00
☐	other than a small entity	$300.00

Appeal Brief fee due $ 150.00

CERTIFICATE OF MAILING/TRANSMISSION (37 C.F.R. § 1.8(a))

I hereby certify that this correspondence is, on the date shown below, being:

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☒ deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Date: 06/22/00

FACSIMILE

☐ transmitted by facsimile to the Patent and Trademark Office.

Tammi L. Taylor
Signature

Tammi L. Taylor
(type or print name of person certifying)

4. EXTENSION OF TERM

NOTE: The time periods set forth in 37 C.F.R. § 1.192(a) are subject to the provision of § 1.136 for patent applications. 37 C.F.R. § 1.191(d). See also Notice of November 5, 1985 (1060 O.G. 27).

NOTE: As the two-month period set in § 1.192(a) for filing an appeal brief is not subject to the six-month maximum period specified in 35 U.S.C. § 133, the period for filing an appeal brief may be extended up to seven months. 62 Fed. Reg. 53,131, at 53,156; 1203 O.G. 63, at 84 (Oct. 10, 1997).

The proceedings herein are for a patent application and the provisions of 37 C.F.R. § 1.136 apply.

(complete (a) or (b), as applicable)

(a) ☐ Applicant petitions for an extension of time under 37 C.F.R. § 1.136 (fees: 37 C.F.R. § 1.17(a)(1)-(5)) for the total number of months checked below:

Extension (months)	Fee for other than small entity	Fee for small entity
☐ one month	$ 110.00	$ 55.00
☐ two months	$ 380.00	$ 190.00
☐ three months	$ 870.00	$ 435.00
☐ four months	$ 1,360.00	$ 680.00
☐ five months	$ 1,850.00	$ 925.00

Fee: $ _____

If an additional extension of time is required, please consider this a petition therefor.

(check and complete the next item, if applicable)

☐ An extension for _____ months has already been secured, and the fee paid therefor of $_____ is deducted from the total fee due for the total months of extension now requested.

Extension fee due with this request $_____

or

(b) ☒ Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

5. TOTAL FEE DUE

The total fee due is:

Appeal brief fee $ 150

Extension fee (if any) $ -0-

TOTAL FEE DUE $ 150

6. FEE PAYMENT

☒ Attached is a check in the sum of $ 150.

☐ Charge Account No. _____ the sum of $_____.

A duplicate of this transmittal is attached.

7. FEE DEFICIENCY

NOTE: If there is a fee deficiency and there is no authorization to charge an account, additional fees are necessary to cover the additional time consumed in making up the original deficiency. If the maximum six-month period has expired before the deficiency is noted and corrected, the application is held abandoned. In those instances where authorization to charge is included, processing delays are encountered in returning the papers to the PTO Finance Branch in order to apply these charges prior to action on the cases. Authorization to change the deposit account for any fee deficiency should be checked. See the Notice of April 7, 1986, *1065 O.G. 31-33.*

☒ If any additional extension and/or fee is required, this is a request therefor and to charge Account No. 13-0610.

AND/OR

☒ If any additional fee for claims is required, charge Account No. 13-0610.

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MSU 4.1-406
06/22/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Alberto L. Mendoza

Serial No.: 09/082,112 Group Art Unit: 1644

Filed : 1998 May 20

For : METHOD AND VACCINE FOR TREATMENT OF PYTHIOSIS INSIDIOSI IN HUMANS AND LOWER ANIMALS

Examiner : S. Turner

Assistant Commissioner For Patents

Washington, D.C. 20231

BRIEF UNDER 37 CFR § 1.192

Sir:

This is an appeal from a final rejection in the above entitled application. The claims on appeal are set forth as Appendix A. An oral hearing will be requested. Enclosed are three (3) copies of this Brief and the fee due upon filing of the Brief.

(1) Real Party in Interest

The real party in interest is Michigan State University, East Lansing, Michigan, a constitutional corporation of the State of Michigan, which is the assignee of the above entitled application.

(2) Related Appeals and Interferences

There are no related appeals or interferences. This application is a divisional of Ser. No. 08/895,940 ('940), which was filed July 17, 1997 and is now U.S. Patent No. 5,948,413. Another divisional of the '940 application, Ser. No. 08/082,232 ('232), was filed May 20, 1998. A Notice of Appeal was filed for the '232 application on January 4, 2000; however, on March 6, 2000 the '232 application was refiled as a Continued Prosecution Application. The '232 application remains pending.

(3) Status of Claims

Claims 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27 are pending. All claims are rejected. Claims 1-15 were canceled in a preliminary amendment which was filed May 20, 1998. Claims 26 and 27 were added by an amendment that was filed September 28, 1999.

(4) Status of Amendments

An Amendment After Final was submitted January 24, 2000 but the amendment was not entered. A second Amendment After Final accompanied by a Declaration under 37 C.F.R. § 1.132 was submitted on March 3, 2000. It is presumed that the second amendment was not entered.

(5) Summary of Invention

The invention provides a protein vaccine for treatment of *Pythium insidiosum* infections in humans and lower mammals. The invention further provides a method for preparing the vaccine which comprises an admixture of separately isolated intracellular antigens from a disrupted *P. insidiosum* cell mass grown in culture and separately soluble extracellular proteins of *P. insidiosum* from the growth medium.

To prepare the vaccine, a culture of *P. insidiosum* is killed with thimersol and the cell mass is removed from the culture fluid to produce a liquid phase which contains the soluble extracellular proteins (exoantigens) of *P. insidiosum* (Specification: page 7, lines 3-7). The cells in the cell mass are disrupted and the disrupted cells centrifuged to produce a supernatant fraction containing the soluble intracellular antigens and a pellet containing the insoluble cell debris which is discarded (Specification: page 7, lines 8-13). The supernatant fraction is then admixed with the liquid phase (Specification: page 7, lines 14-16). Next, the proteins in the admixture are precipitated, the precipitate resuspended in water, and the suspension dialyzed to remove material with a molecular weight less than 10,000 (Specification: page 7, lines 28-35). The dialyzate is the vaccine of the

present invention.

The above vaccine, which contains only the soluble intracellular antigens and the soluble extracellular antigens, was shown to cure horses chronically infected with *P. insidiosum* as well as cure horses that are early in the course of a *P. insidiosum* infection (Specification: page 9, lines 8-14). None of the prior art vaccines are able to cure chronically infected horses (Specification: page 8, lines 13-16). The vaccine was also shown to successfully treat a human with a life threatening case of *Pythiosis insidiosum* arteritis (Specification: page 9, lines 28-31).

(6) Issues

(a) Claims 16-22 and 24-27 were rejected under 35 U.S.C. § 102(b) as being anticipated by Mendoza *et al.* (IDS: AI; Mycopathologia 119: 89-93 (1992)). In particular, it is stated in the rejection that the cell-mass vaccine (CMV) of Mendoza (AI), which contains soluble intracellular antigens and insoluble cell debris prepared from cells which had been removed from the culture fluid, contains the same mixture of soluble intracellular and extracellular antigens as the vaccine of the present invention, and that the soluble concentrated antigen vaccine (SCAV) of Mendoza (AI), prepared from the culture fluid which had been removed

from the cells contains the same mixture of soluble intracellular and extracellular antigens as the vaccine of the present invention.

(b) Claims 16-22 and 24-27 were rejected under 35 U.S.C. § 102(b) as being unpatentable over Mendoza *et al.* (J. Mycol. Med. 6: 151-164 (1996)). In particular, it is stated in the rejection that the Mendoza (1996) vaccine, which is prepared by mixing three particular intracellular antigens with the SCAV vaccine, is the same as the vaccine of the present invention which contains all of the soluble intracellular antigens and soluble extracellular antigens.

(c) Claims 23 and 26-27 were rejected under 35 U.S.C. § 103(a) as being unpatentable over either Mendoza *et al.* (IDS: AI) or Mendoza (1996) in view of Mendoza *et al.* (IDS: AJ; J. Clin. Microbiol. 30: 2980-2983 (1992)) and Panella *et al.* (Cancer Res. 50: 4429-4435 (1990)). In particular, it is stated in the rejection that it would have been obvious for one skilled in the art to make the vaccine of the present invention because Mendoza (AI) teaches CMV and SCAV vaccines, Mendoza (1996) teaches adding three particular intracellular antigens to the SCAV, Mendoza (AJ) teaches the identification of the three particular antigens, and Panella teaches that thimersol affects differentiation of leukemia cells.

(d) The amendment filed October 17, 1999 was objected to under 35 U.S.C. § 132 for allegedly introducing new matter.

(e) Claim 21 was rejected under 35 U.S.C. § 112, first paragraph, for allegedly containing new matter.

(7) Grouping of Claims

Claims 16, 17, and 26, which stand and fall together, are patentably distinct from Claims 18-25 and 27, which stand and fall together. Claims 16, 17, and 26 relate to a method for treating pythiosis in humans and Claims 18-25 and 27 relate to a method for treating pythiosis in a mammal. The claims drawn to treating a human are patentably distinct because while technically humans are mammals, culturally humans are considered distinct and treatments which would be considered appropriate for mammals are generally not considered to be necessarily appropriate in humans.

(8) Argument

(a) Claims 16-22 and 24-27 were rejected under 35 U.S.C. § 102(b) as being anticipated by Mendoza (IDS: AI).

As used in the application and herein, the term "extracellular antigens" means the soluble antigens

which are expressed by *P. insidiosum* and extruded into the culture medium. These antigens, which are not cell-associated, are separate from the antigens inside the cells or attached to the surface of the cells. Therefore, the extracellular antigens as defined by the applicant does not include those antigens which are a part of the cell's outer membrane as implied by the Examiner. As used in the application and herein, the term "intracellular antigens" includes the soluble intracellular antigens contained within the cells and the insoluble antigens associated with the cell debris. The soluble intracellular antigens are prepared by disrupting the cells and removing the insoluble cell debris. If during preparation of the soluble intracellular antigens there are any antigens which are on the outer surface of the cell which are released and become soluble, they will be included with the soluble intracellular antigens. The pending claims are clear as to the distinction the applicant has made between the extracellular antigens and the intracellular antigens. After the soluble extracellular and soluble intracellular antigen fractions are prepared, they are admixed and the antigens in the admixture are precipitated from cell material that is non-precipitable in acetone. The precipitate is resuspended in water and dialyzed to remove low molecular weight materials.

There is no suggestion in any of the prior art to take the above steps in preparing a *Pythiosis* vaccine. It is the applicants vaccine which is shown to provide an unexpected efficacy in the nature of a synergism and shown to be efficacious in curing a human of *Pythiosis*.

In contrast to the vaccine of the present invention, Mendoza (AI) teaches two vaccines, a soluble concentrated antigen vaccine (SCAV) consisting solely of extracellular antigens that are extruded by the cell into the medium, and a cell-mass vaccine (CMV) consisting of both the soluble and insoluble intracellular antigens. Both of these prior art vaccines are distinguishable from the vaccine of the present invention.

The SCAV disclosed in Mendoza (AI) was prepared according to Mendoza and Alfaro in Mycopathologia 94: 123-129 (1986) (IDS: AB) by removing the cells from the medium and then isolating from the medium those antigens that had been extruded by the cells into the medium, i.e., the soluble extracellular antigens. Since the cells were removed from the medium intact, and not disrupted to release soluble intracellular antigens, the SCAV does not contain soluble intracellular antigens. The method disclosed in Mendoza (AI) omitted describing the step of filtering the medium to remove the cells from the medium before

concentrating the medium 20-fold in a stir cell (Mendoza (AI): sentence spanning pages 90-91). However, note that Mendoza (AI) cites on page 90 (right col., para. 2) that it followed the method of Mendoza and Alfaro (*ibid.*) which describes the omitted step. Because the cells are removed from the culture fluid intact, it is clear that the SCAV cannot contain the same complement of antigens as the applicant's vaccine. Thus, the vaccine of the present invention is distinguishable from the SCAV vaccine because the vaccine of the present invention contains both soluble extracellular antigens extruded into the medium and all of the soluble intracellular antigens contained with the cells (specification: page 7, steps 3-6).

The CMV of Mendoza (AI) was prepared from a cell mass that had been washed free of medium containing soluble extracellular antigens. The cell mass was then disrupted to expose the soluble intracellular antigens. After disruption, the cell mass was desiccated overnight to produce a dried composition consisting of soluble intracellular antigens and cell debris containing insoluble intracellular antigens. The dried composition was then dissolved in saline to produce the vaccine. Thus, the CMV consists of both soluble intracellular antigens and insoluble intracellular antigens; it does not contain the soluble extracellular antigens that were

extruded into the medium because the medium was discarded before the cells were disrupted. Therefore, the vaccine of the present invention is distinguishable from the CMV vaccine because the vaccine of the present invention consists of an admixture of soluble extracellular antigens extruded into the medium and soluble intracellular antigens released from the cells after disruption, but not the insoluble intracellular antigens (specification: page 7, steps 4-6).

Since neither the CMV nor the SCAV of Mendoza (AI) contains all of the types of soluble intracellular antigens and the soluble extracellular antigens as does the applicant's vaccine, the CMV and SCAV do not anticipate the present invention under 35 U.S.C. § 102(b). Furthermore, merely combining the prior art vaccines would not provide the vaccine of the present invention because the resulting vaccine would contain insoluble antigens (because the CMV includes the cell debris), material not precipitable by acetone, and material with a molecular weight less than 10,000. While an ultraconcentrator containing a PM-10 filter would remove 10,000 MW material from the culture fluid during preparation of the SCAV, the CMV was not concentrated. Therefore, the CMV would include material with a molecular weight less than 10,000 MW. Thus, the prior art does not anticipate the applicant's vaccine

and, therefore, it does not anticipate the method for treating *Pythiosis*-infected horses as claimed in Claims 18-22, 24-25, and 27, and the method for treating *Pythiosis*-infected humans as claimed in Claims 16-17 and 26.

Finally, Mendoza (AI) relates to vaccines and methods for treating *Pythiosis* in horses with the vaccines. Even if the CMV or SCAV of Mendoza (AI) could be construed to anticipate the applicant's vaccine and its use for treating *Pythiosis*-infected horses as claimed in Claims 18-22, 24-25, and 27, using the applicant's vaccine for treating *Pythiosis*-infected humans as claimed in Claims 16, 17, and 26 would not have been anticipated by Mendoza (AI).

(b) Claims 16-22 and 24-27 were rejected under 35 U.S.C. § 102(b) as being unpatentable over Mendoza (1996).

Mendoza (1996) discloses a vaccine that contains the 28K, 30K, and 32K soluble intracellular antigens added to the "original *Pythium*-vaccine" (Mendoza (1996): page 159, column 2, lines 15-18). The "original *Pythium*-vaccine" is equivalent to the SCAV of Mendoza (AI). While the Mendoza (1996) vaccine contains the 28K, 30K, and 32K soluble intracellular antigens added to the SCAV, not included in the Mendoza (1996)

vaccine are the many other soluble intracellular antigens which are provided by the disruption of the cell mass and are included in the vaccine of the present invention. An abstract dated September 1995, which was submitted with the Amendment After Final filed January 24, 2000, shows that the SCAV was mixed with only the particular isolated intracellular antigens. However, to prepare a vaccine that comprises the SCAV with the isolated 28K, 30K, and 32K antigens is too expensive and thus, of little practical value.

One skilled in the art could not predict the results from the claimed composition which includes all of the soluble intracellular antigens admixed with the soluble extracellular antigens. Particularly, when the non-acetone precipitable material and material less than 10,000 MW is removed as taught by the applicant. Therefore, the vaccine disclosed in Mendoza (1996) is distinguishable from the vaccine of the present invention and does not anticipate the present invention. Thus, the prior art does not anticipates the applicant's vaccine, and, thus, it does not anticipate the methods for using the applicant's vaccine to treat *Pythiosis*-infected horses as claimed in Claims 18-22, 24-25, and 27, and to treat *Pythiosis*-infected humans as claimed in Claims 16-17 and 26.

Finally, even if the vaccine of Mendoza (1996)

could be construed to anticipate the applicant's vaccine and method for treating *Pythiosis*-infected horses as claimed in Claims 18-15, and 27, using the applicant's vaccine for treating *Pythiosis*-infected humans is not anticipated by Mendoza (1996). Mendoza (1996) does provide a method for treating *Pythiosis*-infected humans. Thus, the method for treating *Pythiosis*-infected humans as claimed in Claims 16, 17, and 26, is not anticipated by the vaccine of Mendoza (1996).

(c) Claims 23 and 26-27 were rejected under 35 U.S.C. § 103(a) as being unpatentable over either Mendoza (IDS: AI) or Mendoza (1996) in view of Mendoza (IDS: AJ) and Panella.

By discussing each of the above prior art references separately and showing how the applicant's invention is distinguishable from each before discussing the prior art as a whole will make it clear that the prior art as a whole does not render the applicant's invention obvious.

Mendoza (AI) teaches two different methods for producing *Pythium insidiosum* vaccines, (1) a cell-mass vaccine (CMV) containing both the soluble intracellular antigens and insoluble intracellular antigens from the disrupted-cell debris of *P. insidiosum*, and (2) a soluble concentrated antigen vaccine (SCAV) containing

only the extracellular antigens that are extruded by *P. insidiosum* into the cell culture medium. Both vaccines are effective as immunotherapy vaccines for curing horses that have been infected with *P. insidiosum* for less than 0.5 months (Mendoza (AI): page 92, Table 1). However, these vaccines are of limited efficacy for curing horses that have been infected for greater than 0.5 months but less than 2 months, and neither vaccine is effective for treating horses that have been infected for more than 2 months (Mendoza (AI): page 92, Table 1, and page 93, Tables 3 and 4). Thus, the CMV and SCAV are of similar efficacy. However, Mendoza (AI) teaches that the SCAV is more practical than the CMV because it retains its effectiveness for up to a year of storage after preparation and has a less violent inflammatory reaction at the site of injection than the CMV (Mendoza (AI): page 94, last paragraph). Therefore, Mendoza (AI) concludes that the SCAV can be used as the vaccine of choice in early cases of infection (Mendoza (AI): abstract; page 89, last sentence). Thus, Mendoza (AI) teaches that the CMV and SCAV are equivalent in efficacy but that the SCAV vaccine is preferred because of its longer shelf-life and its lower inflammatory reaction. Finally, Mendoza (AI) recommends that the components of the SCAV responsible for immunity be determined (Mendoza (AI): sentence spanning pages 92-93). This

recommendation implies that the preferred vaccine should contain only those extracellular antigens of the SCAV which are immunodominant. Thus, Mendoza (AI) leads one skilled in the art away from the vaccine of the present invention which contains soluble intracellular antigens and towards a vaccine that consists of one or more soluble extracellular antigens.

Mendoza (AJ) teaches that intracellular preparations of *P. insidiosum* contain at least 20 antigens which are recognized by antisera from infected horses, and that three of these antigens appear to be immunodominant. Mendoza (AJ) does not teach a vaccine containing the immunodominant antigens but suggests that the three immunodominant antigens may be useful for immunotherapy in horses (Mendoza (AJ): Abstract) and as protective immunogens in horses (Mendoza (AJ): page 2982, right col., para. 1, last sentence). To evaluate these antigens in vaccination trials, it is reasonable to assume that one skilled in the art would prepare vaccines that contained various combinations of the above antigens in isolated form. Thus, Mendoza (AJ) leads one skilled in the art towards immunotherapies and vaccines for horses that consists of particular immunodominant antigens isolated from the cell.

Mendoza (1996) is a review article about *Pythiosis*. It is not enabling for *Pythiosis* vaccines

since it does not disclose methods for making or using *Pythiosis* vaccines. Mendoza (1996) states that adding the 28K, 30K and 32K soluble intracellular antigens to the "original *Pythium*-vaccine" enhanced its curative properties (Mendoza (1996): page 159, col. 2, lines 15-18). Adding the 28K, 30K, and 32K soluble intracellular antigens to the SCAV of Mendoza (AI) would not produce the vaccine of the present invention because the vaccine would not contain the other soluble intracellular antigens. Furthermore, it would be prohibitively expensive to produce a vaccine that contained particular antigens that had to be gel purified.

Thus, while Mendoza (1996) teaches vaccines that contain soluble extracellular antigens supplemented with particular soluble intracellular antigens, there is nothing in Mendoza (1996) which would suggest a vaccine that contained all of the soluble intracellular antigens free of the insoluble intracellular antigens. Therefore, when viewed together, Mendoza (AI), Mendoza (AJ), and Mendoza (1996) suggest to one skilled in the art that the preferred vaccine should consist of an admixture of particular soluble intracellular and extracellular antigens. The prior art does not suggest an admixture of all the soluble intracellular antigens free of insoluble intracellular antigens and the soluble extracellular antigens extruded into the medium in which

the admixture has been further purified by acetone precipitation and dialysis to remove material less than 10,000 MW.

Panella teaches that thimersol induces terminal differentiation in leukemic blast cells and accelerated differentiation in normal bone marrow erythroid cell cultures. Panella does not disclose methods for making and using *Pythiosis* vaccines. Panella discloses that thimersol has been used as a relatively nontoxic antimicrobial preservative (Panella: paragraph spanning pages 4433-4434). Thimersol is used by the applicant to kill the *Pythium insidiosum* cells prior to processing the cells for vaccines. In the present invention, the amount of thimersol used to kill the cells was about 0.2 mg per ml of culture (specification: page 7, line 4) which is considerably less than the LD_{50} for mice (120 mg/kg) or humans (2 g) (Panella: page 4433, last sentence). Thus, toxicity of thimersol in the vaccine would not have been a motivating factor for one skilled in the art to dialyze the vaccine (Specification: page 7, lines 34-35. Furthermore, the differentiation effect observed in Panella was in blasts from patients with leukemia and several human leukemia cell lines (Panella: abstract). It is not believed that one skilled in the art would be concerned about thimersol's effect on aplastic anemia in

making a *Pythiosis* vaccine. In addition, it is believed that one skilled in the art would know that the precipitation step which precedes the dialysis step (specification: page 7, lines 28-31) would remove a considerable amount if not all of the thimersol. The purpose of the dialysis step is to remove low molecular weight proteins, salts and sugars used to prepare the medium. Any remaining thimersol would also be removed by the dialysis. Therefore, the teachings in Panella would not have been a motivating influence on one skilled in the art who desired to prepare a *Pythiosis* vaccine. Thus, combining Panella with Mendoza (AI), Mendoza (AJ), and Mendoza (1996) would not have made the present invention obvious.

In contrast to the above prior art vaccines, the vaccine of the present invention is more efficacious than either the CMV or SCAV alone. Unexpectedly, the vaccine of the present invention is able to cure horses that have been chronically infected with *P. insidiosum* for greater than 60 days (Specification: page 8, lines 22-27). The vaccine of the present invention also cures all horses that are acutely infected with *P. insidiosum* (Specification: page 8, lines 32-33). Furthermore, the vaccine of the present invention had cured a human who had been infected with *P. insidiosum* for over 60 days (Specification: page 9, lines 28-31). These unexpected

and remarkable properties of the vaccine of the present invention are in distinct contrast to the CMV and SCAV of the prior art which are only effective against *P. insidiosum* in horses infected for less than 15 days and marginally effective in horses infected for more than 45 days.

It is speculation that a vaccine which was prepared by admixing the soluble antigens of the SCAV with the soluble antigens isolated from the CMV and then further prepared by removing the non-acetone precipitable material and material less than 10,000 MW from the admixture would provide a vaccine with the same remarkable properties as the vaccine of the present invention. Thus, the vaccine of the present invention is not a merely an admixture of SCAV and CMV prepared according to the prior art. It would have been unexpected and unobvious that isolating the soluble intracellular antigens from the insoluble intracellular antigens (antigens associated with the cell debris) and then admixing the soluble intracellular antigens with the soluble extracellular antigens extruded into the medium followed by acetone precipitation and dialysis would produce a vaccine with the unexpected and remarkable properties of the vaccine of the present invention, i.e., curing chronically infected horses and humans infected with *Pythiosis*.

As stated in *In re Vaeck*, 20 USPQ2d 1438, at 1442 (Fed. Cir 1991),

> [w]here claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art they should make the claimed composition . . . ; and (2) whether the prior art would also have revealed that in so making . . . , those of ordinary skill would have a reasonable expectation of success . . . .

In light of *In re Vaeck*, the prior art references do not suggest nor imply that the vaccine of the present invention would provide a more efficacious vaccine. Since Mendoza (AI) teaches that neither the CMV consisting of all intracellular antigens (both soluble and insoluble) nor the SCAV consisting of extracellular antigens are able to cure infected horses after 60 days or more of infection, there is no reason for one skilled in the art to believe that an admixture of only the soluble intracellular antigens and soluble extracellular antigens made according to the applicant's teachings would produce a vaccine that would cure infected horses 60 days or more after infection. In particular, the inability of either prior art vaccine to cure infected horses after 60 days or more of infection and the preference of Mendoza (AI) for the SCAV consisting of extracellular antigens teaches away from the present

invention. Mendoza (1996) teaches that a modified vaccine containing the 28K, 30K, 32K intracellular antigens had enhanced curative properties which implies that the intracellular antigens in a vaccine should be isolated from the cells, not that a mixture of all the soluble intracellular antigens from the cell be used to make the vaccine. Finally, Mendoza (AI) teaches that the immunogenic antigens in the SCAV be identified, ostensively for vaccine use (Mendoza (AI): sentence spanning pages 92-93). Therefore, it would not be reasonable to expect one skilled in the art to admix the total soluble intracellular antigens and total soluble extracellular antigens as taught by the applicant to make the vaccine of the present invention absent some teaching in the prior art which would suggest that doing so would produce a more efficacious vaccine. Furthermore, in view of the prior art, one skilled in the art would have little motivation to produce the vaccine of the present invention because to do so would entail increased effort to produce a vaccine with an expected efficacy no better than the efficacy of either prior art vaccine. Finally, Panella, which discloses that thimersol can induce leukemic cells to differentiate, is not expected to have had any motivating influence on one skilled in the art in preparing a *Pythiosis* vaccine. That is because, in

general, thimersol is considered to be a relatively non-toxic antimicrobial preservative, particularly at the levels used by the applicants to kill the cells.

Also *In re Vaeck* stands for the proposition that in demonstrating obviousness it should be established that one skilled in the art would have had a reasonable expectation of success in making the present invention. In the present case, the prior art provides no indication that combining only the soluble intracellular antigens isolated from the CMV with the soluble extracellular antigens of the SCAV in which material non-precipitable by acetone and material less than 10,000 MW are removed would produce a vaccine of unexpected enhanced efficacy, i.e., a vaccine with the ability to cure horses infected for more than 60 days and cure humans infected with *Pythiosis*. At best, one skilled in the art would expect the prior art to produce a vaccine that may be as efficacious as either prior art vaccine, but not a vaccine with the unexpected properties of the vaccine of the present invention. In view of Mendoza (AI), one skilled in the art may even expect that the vaccine would be less desirable, since Mendoza (AI) shows that the intracellular antigen (CMV) vaccine is less stable and more inflammatory than the extracellular antigen (SCAV) vaccine (Mendoza (AI): page 94, last paragraph). In view of Mendoza (1996), one

skilled in the art may at best expect an SCAV that further included the isolated 28K, 30K, and 32K intracellular antigens to have enhanced curative properties. However, Mendoza (1996) does not disclose what these enhanced curative properties are. Also, since Mendoza (1996) teaches that the total intracellular antigen vaccines were less stable (Mendoza (1996): page 161, column 1, second paragraph), one skilled in the art would not expect that a vaccine that contained the total soluble intracellular antigens as claimed in the present invention to have the enhanced curative properties eluded to in Mendoza (1996).

Subsequent to *In re Vaeck*, the court has consistently held that absent some teaching or suggestion that would support combining the prior art references, obviousness cannot be established by merely by combining the teachings of the prior art to make the applicant's invention. *In re Bell*, 26 USPQ2d 1529 (Fed. Cir. 1993), *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988), and *ACS Hospital Systems v. Montefiore Hospital*, 221 USPQ 929 (Fed. Cir. 1984). In the present rejection, not only must the teachings of the prior art be combined, but superimposed on the combined prior art must be included the further steps taught by the applicant but not taught by the prior art, i.e., removing cell debris (insoluble antigens), removing non-

acetone precipitable material, and removing material less than 10,000 MW. Thus, it appears that the present rejection is a hindsight reconstruction of the invention from the applicant's own disclosure, which is not permitted. As stated in *In re Vaeck*, at 1442, "[b]oth the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure." In the present rejection, the suggestion and motivation for combining the soluble extracellular antigens with the soluble intracellular antigens in the process taught by the applicant to make the vaccine of the present invention becomes obvious only in view of the applicant's disclosure, which teaches that the combination when prepared as taught produces a vaccine with enhanced efficacy. In the absence of the applicant's disclosure, the prior art teaches that the CMV and SCAV are equivalent in efficacy, and that the SCAV is preferred over the CMV because of its longer shelf-life and its lower inflammatory reaction at the site of injection. Further, the art teaches that the most preferred vaccine consists solely of particular intracellular antigens and extracellular antigens (Mendoza (1996): page 159, col. 2, lines 15-18). There is nothing in the prior art to suggest to one skilled in the art that admixing the total soluble intracellular antigens of the CMV with the total soluble extracellular

antigens of the SCAV would produce a vaccine of enhanced efficacy, much less in the manner taught by the applicant. It is only the applicant's disclosure that teaches that a vaccine comprising both the soluble intracellular antigens and soluble extracellular antigens prepared in the manner taught by the applicant produces a vaccine with the unexpected ability to cure horses infected for more than 60 days and humans infected with *Pythiosis*. It is mere speculation that merely combining the SCAV and CMV would produce an equivalent vaccine. Therefore, unless one skilled in the art had access to the applicant's disclosure, it would not have been obvious to make the vaccine of the present invention.

Mendoza (1996) cannot substitute for the applicant's disclosure because Mendoza (1996) teaches a vaccine containing soluble extracellular antigens and three immunodominant intracellular antigens to provide a vaccine with some undisclosed but enhanced curative properties. It would be overly speculative to construe that the enhanced curative properties of the vaccine in Mendoza (1996) would have led one skilled in the art to expect that a vaccine similar to the vaccine of the present invention would be as "enhanced" (or more "enhanced") as the vaccine of Mendoza (1996). Therefore, because none of the prior art references can

sustain an obviousness rejection either alone or in combination, it appears that the present rejection can only be sustained in view of the applicant's disclosure which is a hindsight reconstruction that is not permitted.

Finally, even if the prior art could be interpreted to render the applicant's vaccine and method for using it to treat *Pythiosis*-infected horses as claimed in Claims 23 and 27, the prior art does not render obvious the applicant's vaccine for treating *Pythiosis* in humans as claimed in Claim 26. *Pythiosis* in humans is a disfiguring and life-threatening disease (Specification: pages 11-12). The prior art does not suggest nor support the proposition that a vaccine which can be used to treat *Pythiosis*-infected horses could be used to treat P*ythiosis*-infected humans. While one skilled in the art would be motivated to find a cure for humans infected with *Pythiosis*, it would not have been obvious that combining particular elements of the prior art vaccines, which were known to have limited efficacy in chronically infected horses, and use the resulting vaccine, to treat humans, particularly a chronically infected boy. In particular, it would not have been obvious that the dialysis, which removed material less than 10,000 MW, would have been sufficient to make the vaccine safe or effective in humans, despite the showing

of its effectiveness in chronically infected horses. In general, vaccines which are used to vaccinate animals are not used to vaccinate humans and treatments which are effective in animals are not necessarily effective in humans. This is reflected by the fact that even though various vaccine formulations had been shown to be effective in curing *Pythiosis*-infected horses, the treatment of the *Pythiosis*-infected boy consisted of various antifungal drugs. However, the antifungal drugs were ineffective and as a last resort, the boy was vaccinated with the applicant's vaccine (Specification: page 13, lines 7-11). Therefore, it was remarkable when it was found that the applicant's vaccine, without modification for human use, was able to cure a boy infected with *Pythiosis* (Specification: page 15, lines 17-31). Thus, the method for treating *Pythiosis*-infected humans as claimed in Claim 26, is not obvious in view of the prior art.

For the above reasons, it is believed that the prior art does not make the present invention obvious. Therefore, reconsideration of the rejection is requested.

(d) The amendment filed October 17, 1999 was objected to under 35 U.S.C. § 132 because it introduces new matter.

The applicants had made two deposits of *Pythium insidiosum* CBS 574.85, a non-Budapest Treaty deposit designated ATCC 58643 and a Budapest Treaty deposit designated ATCC 74446 which is a redeposit of ATCC 58643 made June 1, 1998. The specification incorrectly provided the ATCC designation for the non-Budapest Treaty deposit (Specification: page 5, lines 19-26). ATCC 74446 was identified on the ATCC Budapest Treaty deposit certificate as *P. insidiosum* CBS 574.85. *P. insidiosum* CBS 574.85 was identified as ATCC 58643 in Mendoza (AI) on page 90, column 2, line 15. A statement of Deposit identifying ATCC 74446 as CBS 574.85 was enclosed with the amendment filed October 5, 1999. Enclosed are ATCC Internet catalog descriptions for ATCC 58643 and ATCC 74446 which further show that ATCC 74446 is a redeposit of ATCC 58643. Thus, the *P. insidiosum* of ATCC 74446 is identical to the *P. insidiosum* of ATCC 58643 and is not new matter. The amendment filed October 17, 1999 corrected the incorrect ATCC designation wherever it occurred in the application. In light of the above, the amendment filed October 17, 1999 does not introduce any new matter into the specification.

(e) Claim 21 was rejected under 35 U.S.C. § 112, first paragraph, as containing new matter.

As discussed in (d) above, no new matter had been introduced into Claim 21 by the amendment filed October 17, 1999, which deleted "58643" in ATCC 58643 and inserted "74446" therefor.

(9) Conclusion

For the above reasons it is believed that Claims 18 to 25 and 27, which relate to a method for treating *Pythiosis*-infected horses with applicant's vaccine, are patentable, and Claims 16, 17, and 26, which relate to a method for treating *Pythiosis*-infected humans with applicant's vaccine, are patentable. Reconsideration of the Examiner's rejections and objections is requested. Allowance of Claims 16 to 27 is requested.

Respectfully,

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Registration No. 20,931

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Encls.: Catalog description for ATCC 74446
Catalog description for ATCC 58643

APPENDIX A

-16-

A method for treatment of Pythiosis in human patients having the disease which comprises:

(a) providing a vaccine containing a mixture of proteins of *Pythium insidiosum* in a sterile aqueous solution, wherein the mixture of proteins is (1) of mixed intracellular proteins removed from disrupted cells of the *Pythium insidiosum* grown in a culture medium and (2) of mixed extracellular proteins from the culture medium for growing the *Pythium insidiosum*; and

(b) vaccinating the patient with the vaccine.

-17-

The method of Claim 16 wherein the vaccination is subcutaneous.

-18-

A method for the treatment of Pythiosis in a mammal having the disease which comprises:

(a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum* in a culture medium which comprises in a sterile aqueous solution in admixture:

(1) mixed intracellular proteins removed from disrupted cells of the *Pythium insidiosum*; and

(2) mixed extracellular proteins removed from a supernatant from growing the cells of the *Pythium insidiosum*; and

(b) vaccinating the mammal with the vaccine.

-19-

The method of Claim 18 wherein the removed proteins have been provided by growing cells of the *Pythium insidiosum* in the culture medium, then killing the cells, then separating the killed cells from the culture medium to produce a first supernatant containing the mixed extracellular proteins and then disrupting the killed cells in sterile water to provide the mixed intracellular proteins in a second supernatant and removing the mixed intracellular proteins from the disrupted cells and removing the mixed extracellular proteins from the first supernatant.

-20-

The method of Claim 18 wherein the cells have been disrupted by sonication.

-21-

The method of Claim 18 wherein the *Pythium insidiosum* is deposited as ATCC 74446.

-22-

The method of any one of Claims 19, 20 or 21 wherein the culture medium is Sabouraud's dextrose broth.

-23-

The method of Claim 19 wherein the cells are killed with thimersol.

-24-

The method of Claim 19 wherein the disrupted cells are removed from the culture medium for the cells by centrifugation to provide the mixed intracellular proteins in the second supernatant.

-25-

The method of Claim 19 wherein the removed proteins have been precipitated together from a combined mixture of the first and second supernatants using acetone and then isolated and dispensed in sterile distilled water to provide the vaccine.

-26-

The method of Claim 16 wherein the mixed intracellular proteins and mixed extracellular proteins in water have been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine.

-27-

The method of Claim 18 wherein the mixed intracellular proteins and mixed extracellular proteins in water has been dialyzed to remove low molecular weight components less than 10,000 MW to produce the vaccine.

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74446 Word Search Clear Search

For query options, please read the search help.

Fungi, Yeasts, & Yeast Genetic Stock	
ATCC Number:	**74446** order this item
Organism:	*Pythium insidiosum* de Cock et al.
Designation:	H-9 [CBS 574.85]
Depositors:	Michigan State Univ.
Subcollection:	Fungi
Isolation:	horse with pythiosis and cutaneous granuloma, Costa Rica [IV87997]
Descriptions:	redeposit of ATCC 58643
References:	IV87997: Michigan State Univ. , personal communication
Propagation:	ATCC medium: 307 Cornmeal agar (Difco 0386) Temperature: 37C
Patent Statement:	This material is cited in a U.S. and/or other Patent and may not be used to infringe the patent claims.
BioSafety Level:	2
Required Forms:	USDA permit (VS Form 16-3)
Shipped:	test tube, allow 3 weeks for delivery
Price:	$130
Revised :	Jan 07, 2000

Pricing Note:
All prices are in U.S. dollars and are for U.S. Profit & Foreign Institutions. Our domestic (U.S. and Canada) non-profit customer-pricing discount has also changed and is now a standard 20% discount off the list price of most cultures.

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58643 Word Search Clear Search

For query options, please read the search help.

Fungi, Yeasts, & Yeast Genetic Stock	
ATCC Number:	58643 order this item
Organism:	*Pythium insidiosum* de Cock et al.
Designation:	H-9 [CBS 574.85; CDC B-4296]
Depositors:	A.A. Padhye
History:	ATCC <<– Depositor <<– L. Mendoza
Subcollection:	Fungi
Isolation:	horse, Costa Rica
Type Strain:	type strain [RF22372]
Descriptions:	life cycle [RF27716]
References:	RF22372: De Cock AW et al. Pythium insidiosum sp. nov., the etiologic agent of pythiosis. J. Clin. Microbiol. 25: 344-349, 1987 PubMed: 87138250 RF27716: Mendoza L et al. Life cycle of the human and animal oomycete pathogen Pythium insidiosum [published erratum appears in J Clin Microbiol 1994 Jan;32(1):276]. J. Clin. Microbiol. 31: 2967-2973, 1993 PubMed: 94086797
Propagation:	ATCC medium: 307 Cornmeal agar (Difco 0386) Temperature: 24C
BioSafety Level:	2
Shipped:	test tube, allow 3 weeks for delivery
Price:	$130
Revised :	Jan 07, 2000

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Shipping Charges
All materials are shipped FOB Manassas, freight prepaid via carrier of our choice and added to your invoice. Packaging is extra.

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